New High Performance Magnet Structures for Bead Based Molecular Separation

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Abstract

High performance Hybrid magnetic separation technology is under continuing development at the D.O.E. Joint Genome Institute and Lawrence Berkeley National Laboratory for general laboratory and high throughput automated applications. This technology has broad applicability for molecular separation in genomics, proteomics and other areas. It's applicability ranges from large and small scale microtiter plate and flow separation processes to single molecule DNA manipulation. It is currently an enabling purification technology for very high throughput production sequencing at the D.O.E. Joint Genome Institute. This technology incorporates hybrid magnetic structures that combine linear permanent magnet material and ferromagnetic material to produce significantly higher fields and gradients than those of currently available commercial devices. These structures incorporate ferromagnetic poles that can be easily shaped to produce complex field distributions for specialized applications. The higher maximum fields and strong gradients of the hybrid structures result in greater holding forces on magnetized targets that are being processed as well as faster extraction. Current development versions of these magnet plates have exhibited fields in excess of 1.0 tesla and gradients approaching 1000.0 tesla/meter. Second generation Hybrid magnet plates have now been developed for both 384 and 96-well applications. This technology is currently being made available to industry through the Tech Transfer Department at Lawrence Berkeley National Laboratory.

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HED: New High-performance Magnet Structures for Bead-based Molecular Separation
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Breakthrough Technology with Wide Applicability
High-performance hybrid magnetic separation technology is in use and under continuing development at the D.O.E. Joint Genome Institute (JGI) (Walnut Creek, CA) and Lawrence Berkeley National Laboratory (LBNL) (Berkeley, CA) for general laboratory and high throughput automated magnetic bead-based applications. This technology has broad applicability for molecular separation in genomics, proteomics and other areas, with usage ranging from large- and small-scale microtitre plate and flow separation processes to microarrays and single molecule DNA manipulation. Magnet plates incorporating this technology, such as the one shown in Figure 1, have exhibited fields in excess of 1.1 tesla (11,000 gauss).

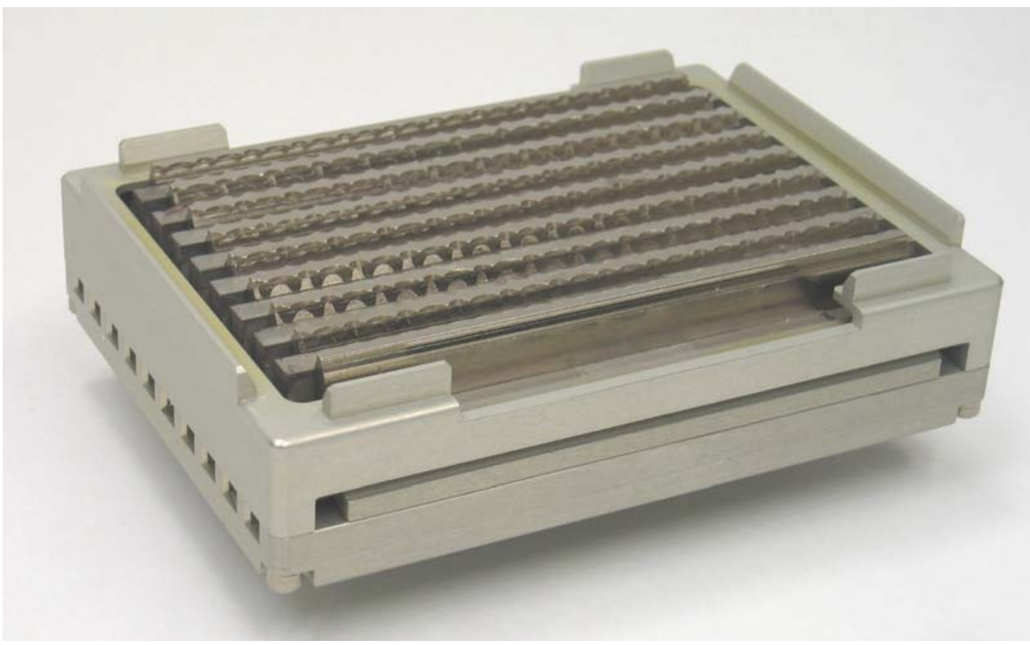

Figure 1: The second-generation hybrid-96 magnet plate generates peak fields exceeding 11,000 gauss. Special shaping of the ferromagnetic pole tips for beneficial distribution of magnetized target species in 96-well microtitre plates can be seen in the upper part of the structure. Interchangeable upper microtitre plate interfaces can accommodate a variety of plate types and automation platform requirements.

Bead-based magnetic separation technologies are referred to by various names such as magnetic purification, magnetic assays and solid-phase reversible immobilization. Considerable work has been done on the magnetic bead side of the technology and high-quality beads with increasingly sophisticated affinity coatings are available from a number of manufacturers. The other essential part of magnetic separation is the magnet technology that generates the magnetic fields that act on the beads. This side of separation technology has not kept up with the pace of bead development until recently, with the advent of hybrid magnetic technology.

Commercial versus hybrid magnet plates

Currently available commercial magnet plates are generally based on pure permanent magnet designs that are relatively economical to produce but intrinsically limited in their performance. The peak fields of such plates range from 2.5 to approximately five kilogauss. Hybrid magnet plates produced at LBNL, and in use at JGI, generate peak fields in the range of 8,000 to 11,000 gauss. Some commercial plates and LBNL hybrids are compared in Figure 2. Field strengths of the LBNL hybrids range from more than 100% to 300% higher than those of measured commercial plates.

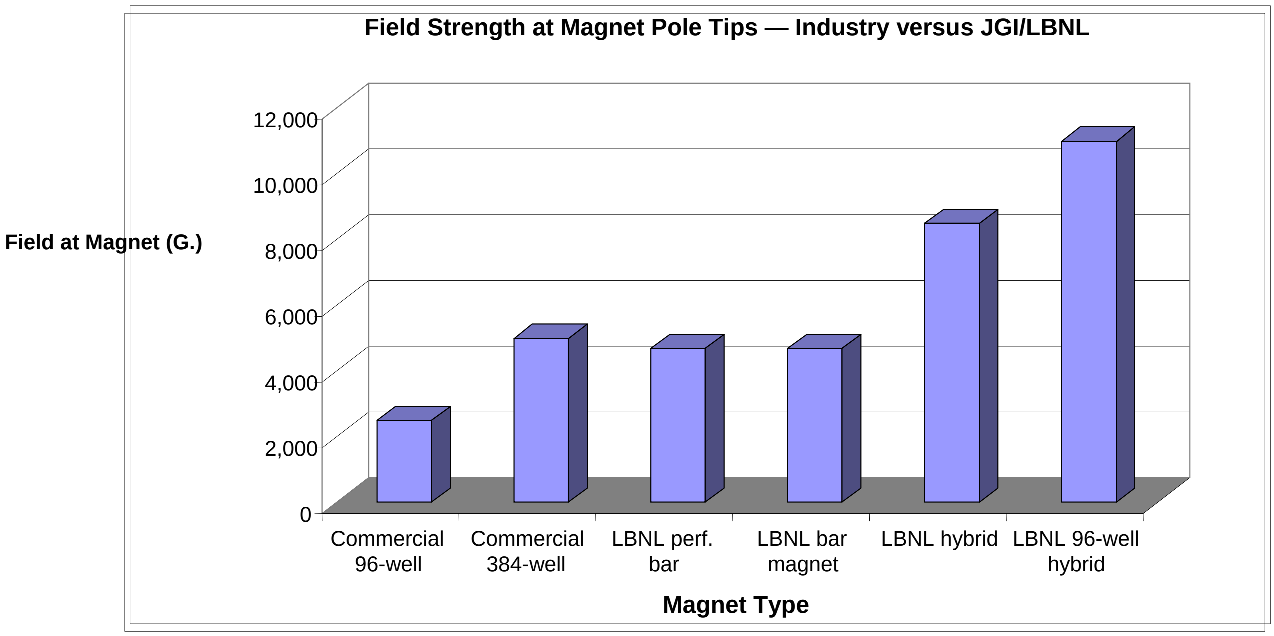

Figure 2: Comparison between commercial magnet plates and LBNL hybrids. Fields are from Hall probe measurements at LBNL. The first two bars from the left illustrate the general range of commercial pure permanent magnet plates. The two centre bars are pure permanent magnet plates developed at LBNL, and the last two bars are LBNL hybrids.

The principal difference between hybrid magnetic structures and other designs is that the hybrid type utilizes both permanent magnet material and ferromagnetic material in a sophisticated arrangement that increases the magnetic flux density in important regions of the magnetic system. The permanent magnets in this system act as the driving or flux-generating elements of the structure. At the same time, the ferromagnetic poles guide and concentrate the flux into the magnetic circuit areas where high fields are needed to affect the magnetic beads that are being acted on in the microtitre plate wells.

Figure 3 illustrates this mechanism. It is a computer model of a partial cross-section of a hybrid magnet plate. The field lines shown are lines of constant vector potential from Maxwell's equations. In such a model, the density of the lines is directly proportional to the strength of the field. As illustrated, the lines of flux are flowing from the permanent magnet blocks into the vertical poles of the structure. Following the lines upward in the pole toward the business region at the trapezoidal pole tips reveals a dramatic increase in the density of the lines, and therefore an increase in the flux density or field strength where the structure interacts with the microtitre plate wells.

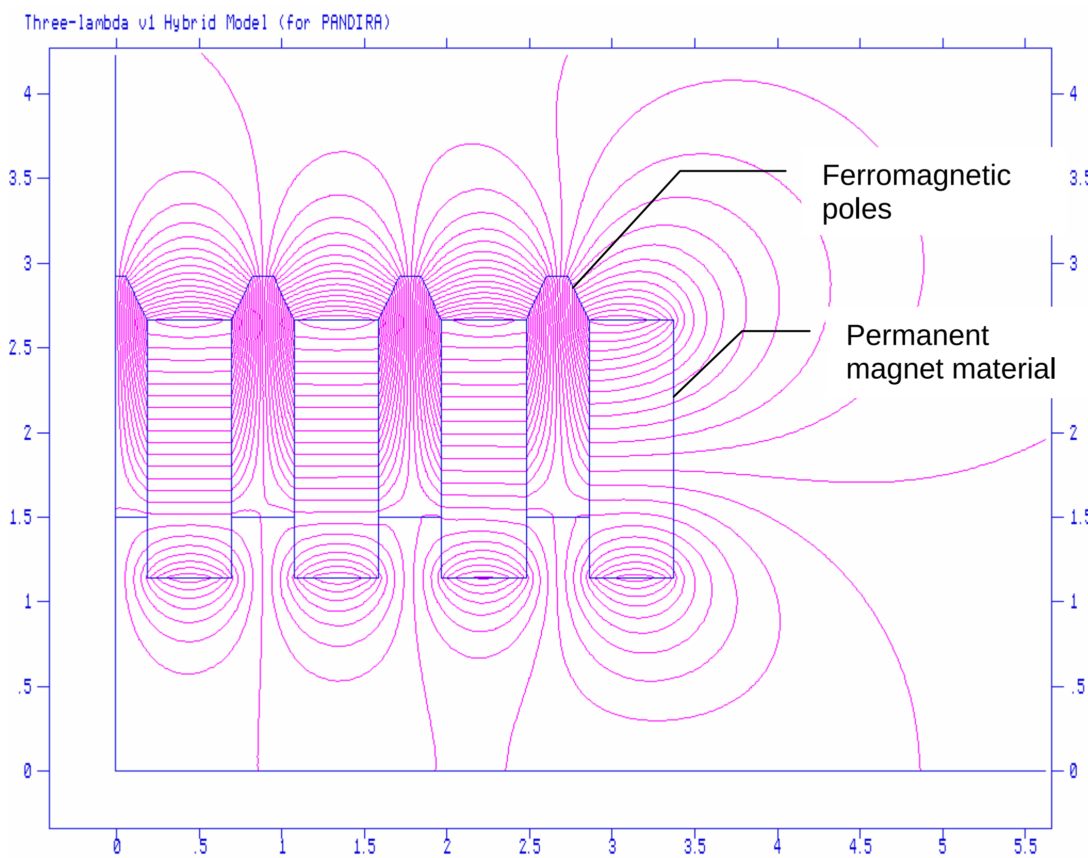

Figure 3: Computer model of hybrid magnetic structure showing distribution of magnetic flux and flux density. This is a cross-section of the magnetic structure of the magnet plate of Figure 4.

Flux density versus gradient

To generate a force on a magnetic bead, field strength and a gradient in the field are required; that is, the field must be changing. If a magnetic bead is in a very strong field but it is uniform (no gradient), then the bead will not experience any force other than an orienting force. The translating force on the bead that pulls it out of the solution in which it is suspended is a function of the product of the field strength and the gradient.

The field strength, or flux density, is an important part of this equation because magnetic beads have what is called a B-H curve that determines the force they experience in a magnetic field with a given gradient. As the field increases, for a fixed gradient, the force on the bead will increase up to a point called the “maximum magnetization” of the bead. This effect becomes increasingly important for beads that are relatively far from the magnetic structure where the fields are decaying — for instance, higher up in the wells of a microtitre plate.

The higher fields of hybrid structures allow greater extension of the fields into the upper reaches of microtitre plate wells to pull down targets faster. The strong gradients at the pole tips of the structure provide greater holding power for targets that have been drawn down to accumulation regions of the wells. In general, this results in a faster, more robust sample process.

Another advantage of ferromagnetic poles is that complex shapes can be easily machined into them for specialized functions and for use with many different types of microtitre plates of varying well shape.

Hybrid Applications to DNA Sequencing

Hybrid technology is currently an enabling sample prep technology for very high throughput production DNA sequencing at JGI. The hybrid-384 magnet plate shown in Figure 4 is of the type used in a critical automated purification process prior to actual sequencing of approximately 2.5 gigabases of DNA per month. It is also used in smaller scale R&D applications for various microtitre plate formats.

Applications involving 1,536-well microtitre plates can be accommodated by hybrid structures with a period length that is half that of the hybrid-384 shown in Figure 4. A prototype of such a structure has been built at LBNL.

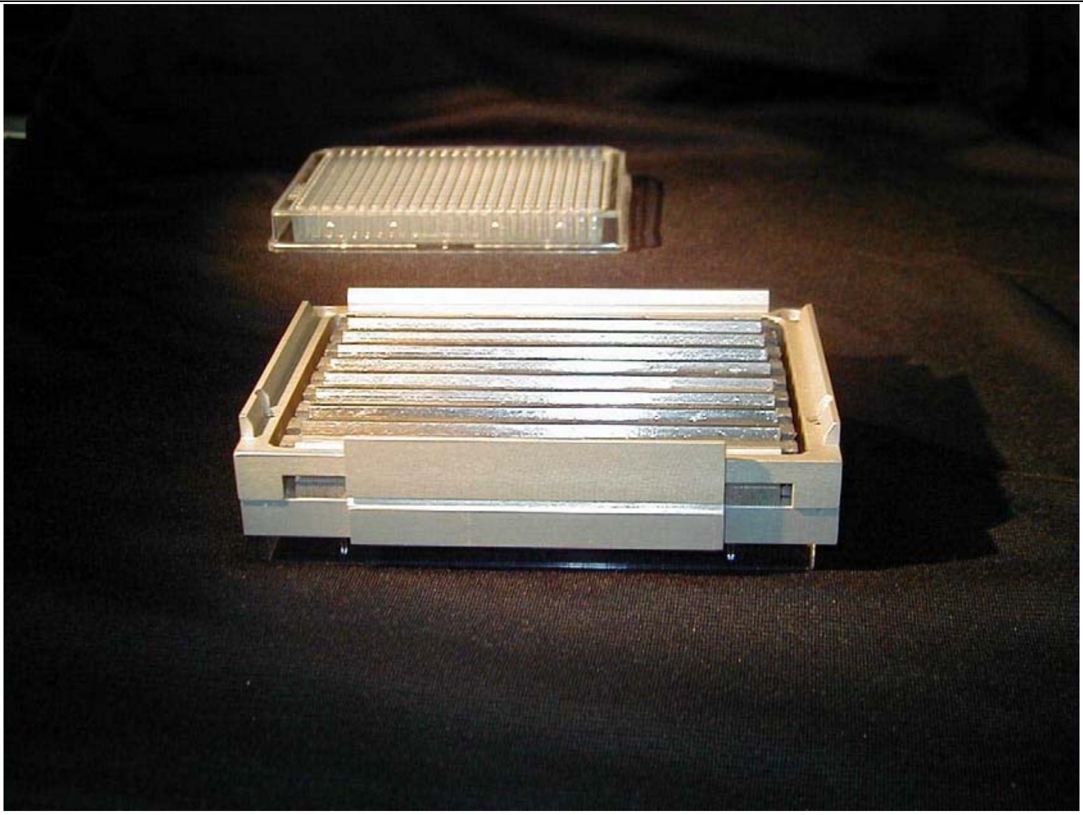

Figure 4: Hybrid-384 magnet plate with 384 square-well microtitre plate in the background. This structure has simple trapezoidal pole-tip shapes that work well with both the flat-bottomed plate shown and 384-well thermal cycler plates.

Other Hybrid Applications
In addition to established production DNA sequencing and R&D purification applications, an increasing number of new and interesting applications for hybrid technology are presenting themselves.

Proteomics
Hybrid technology has clear applicability in the rapidly expanding proteomics arena. A number of hybrid-based, selective separation and extraction methods are currently being explored.

Microarrays
Hybrid technology has the potential to greatly improve the yields of microarray processes. The 1,536-well hybrid plate mentioned previously has 2.25-mm magnetic periodicity along one axis and arbitrarily short periodicity, by virtue of continuity, along the other axis. As a result, short period hybrid variants can be productively applied to high-density microarrays.

Single molecule manipulation

Significant work has been done on hybrids for single molecule manipulation and a separate LBNL patent process has been initiated for this technology. A variety of small-scale hybrid devices have been built and tested. This variant of hybrid technology shows great promise of becoming a major tool for manipulation of DNA, RNA and proteins. It has notable advantages over laser capture technologies and has demonstrated the potential to produce forces on single molecules in the range of zero to 1,000 pico-newtons.

Manipulation of therapeutic nanoparticles

Hybrid technology for therapeutic nanoparticle treatments, such as those for cancer, is in conceptual stages and is another area in which hybrid technology is likely to be beneficially applied to concentrate drug-bearing nanoparticles at tissue treatment sites in living subjects.

In general, any magnetic bead-based purification or manipulation method is likely to benefit from the intrinsic high performance of hybrid magnetic technology.

Technology Transfer Opportunities for Hybrid Technology

Hybrid magnetic technology is being patented by the University of California (Berkeley, CA) and LBNL. This technology is currently being made available for transfer to industry through the Technology Transfer Department of LBNL. In addition to its other areas of expertise, LBNL is evolving as a centre for advanced magnetic R&D for genomic, proteomic and biomedical applications and welcomes collaborative development efforts with industry partners as part of its R&D and technology transfer process.

Acknowledgment

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David Humphries (DEHumphries@LBL.gov) is a staff engineer at Lawrence Berkeley National Laboratory and the D.O.E. Joint Genome Institute. For additional information about hybrid technology, go to: *www.lbl.gov/Tech-Transfer/techs/lbnl1714.html.*

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